Protection Branch Report of Test No. 22-60

Effect of Dry Heat Upon Dry Bacterial Spores

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The sterilizing effect of dry heat was determined at temperatures lower than those usually employed for this purpose, but at longer exposure times. Small cotton plugged glass tubes containing dry spores of Bacillus subtilis var niger mixed with diatomaceous earth, a convenient extender, were subjected to dry heat for various time periods at the Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California. At their request this laboratory assayed the various tubes for viable bacterial spores. The results of the bacteriological assay are the subject of this report.

MATERIALS AND METHODS

Three tubes about 1/3 inch diameter containing approximately 50 milligrams of dry B. subtilis var niger spores and diatomaceous earth were subjected to each of nine test conditions (see Table I for the specific conditions). Two milliliters of sterile tryptose broth were added to each tube and mixed. The first tube of each set was assayed by the pour plate method to determine the number of viable spores in the tube. The other two tubes in each set were incubated at 37 C to test for presence of viable spores. The broth tubes were incubated 7 days when corresponding pour plates showed no growth within 48 hours; then an aliquot was spread on agar to confirm sterility.

RESULTS

Heating for 36 hours at 105 C was not sufficient to kill all the dry spores mixed with diatomaceous earth (Table I). Moreover, not all the spores were killed at 125 C for six hours either. The isolated colony found on some of the spread plates probably was airborne contamination. From these results, it would appear that dry spores will be killed within 12 hours when heated at 125 C, but to sterilize materials that are poor heat conductors, a longer exposure time to heat probably would be required to assure that the interior of the material was subjected to 125 C temperature for 12 hours.

The Effect of Dry Heat Upon Dry Spores of *Bacillus subtilis* var *niger* Mixed with Diatomaceous Earth

Test Conditions	Tube 1 Av Spores/tube	Tube 2 Appearance in Broth	Tube 2 Agar Spread Plate	Tube 3 Appearance in Broth	Tube 3 Agar Spread Plate
Not Heated	3.5×10^7	Cloudy with orange pellicle		Cloudy with orange pellicle	
6 hrs at 105 C	2.6×10^6	Cloudy with orange pellicle		Cloudy with orange pellicle	
12 hrs at 105 C	2.8×10^6	Cloudy with orange pellicle		Cloudy with orange pellicle	
36 hrs at 105 C	2.2×10^3	Cloudy with orange pellicle		Cloudy with orange pellicle	
6 hrs at 125 C	0	Cloudy with orange pellicle		Slightly cloudy	Abundant White Growth*
12 hrs at 125 C	0	Slightly cloudy	No growth	Slightly cloudy	1 "cocci" colony
24 hrs at 125 C	0	Slightly cloudy	1 BSN colony	Slightly cloudy	No growth
36 hrs at 125 C	0	Slightly cloudy	No growth	Slightly cloudy	1 BSN colony
48 hrs at 125 C	0	Slightly cloudy	1 "cocci" colony	Slightly cloudy	No growth

BSN - *Bacillus subtilis* var *niger* colony
"Cocci" Colony - Orange colony showing cocci in clusters when stained with methylene blue.
* A methylene blue stain of the growth showed the presence of bacilli and spores.
Note: All samples that had an orange pellicle displayed typical growth of *B. subtilis* var *niger*; a methylene blue stain of the broth showed the presence of bacilli and spores.